Regioselective Syntheses of 2,3,4-Tribromopyrrole and 2,3,5-Tribromopyrrole

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2,3,4-Tribromopyrrole (**1**) and 2,3,5-tribromopyrrole (**2**) were each synthesized from pyrrole. Spectral data and antifeedant effects for synthetic **1** and the antipredatory chemical defense compound of the marine hemichordate *Saccoglossus kowalevskii* were in agreement, confirming the structure of the deterrent natural product as **1**. Spectral data for **2** differed from synthetic and natural **1**.

Recently, the antipredator chemical defense of a marine hemichordate worm, *Saccoglossus kowalevskii*, was identified as 2,3,4-tribromopyrrole (**1**).[1] At its natural concentration of 0.20% of dry mass, this natural product deterred feeding by two species of predatory fish, *Fundulus heteroclitus* and *Leiostomus xanthurus*, but had no effect on the feeding behavior of a predatory crab, *Callinectes similis*.[1] Although **1** was previously reported from this and other worm species,[2−5] it remained possible that the natural product was in fact 2,3,5-tribromopyrrole (**2**), because of ambiguities in the published spectral data. Specifically, the chemical shift of the methine proton was previously reported using different solvents for **1** and **2**, no ^{13}C NMR spectral data were available for **2**, and mass spectral data were identical for both compounds.[3,4,6] Although the syntheses of **1** and **2** had been achieved from pyrrole (**3**) as a complex mixture,[3] the uncertainties above made it difficult to exclude either structure for the natural product. In this study, we performed a regioselective synthesis of **1** and compared spectral data and feeding assay results for synthetic **1** with data for the tribromopyrrole natural product from *S. kowalevskii*. We also synthesized **2** for comparison of spectral data with **1**.

(1)

(2)
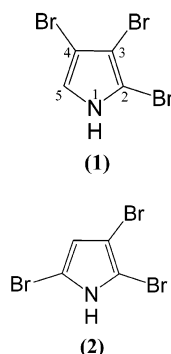
The synthesis of 2,3,4-tribromopyrrole (**1**) was accomplished in three steps from pyrrole (**3**) (Figure 1). We hypothesized that the steric bulk of a silyl protecting group would lead to selective bromination at sites more distant from the protected nitrogen. Protection of **3** afforded the silyl ether **4**,[6] which was then treated with *N*-bromosuccinimide (NBS) at low temperature. Conversion under kinetic control to 2,3,4-tribromo-1-(triisopropylsilyl)pyrrole
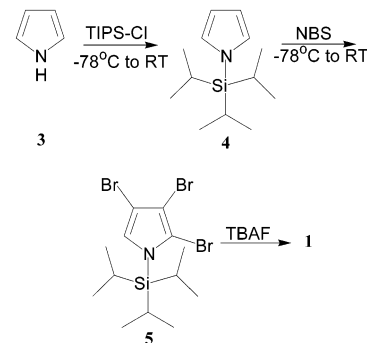
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Figure 1. Synthesis of 2,3,4-tribromopyrrole (**1**).

(**5**) was tracked by LC-MS, and product purification was achieved by recrystallization. Although the bromination step was previously reported to require only one addition of between 3 and 5 equiv of NBS,[6] in our hands, two separate additions provided higher yield (97%) of tribrominated product, and tetrabromination was never observed. Deprotection of **5** was achieved using tetrabutylammonium fluoride (TBAF) under ambient conditions to produce 2,3,4-tribromopyrrole (**1**), for a deprotection yield of 90% and an 87% overall yield of the three-step sequence.

2,3,5-Tribromopyrrole (**2**) was synthesized directly from pyrrole (**3**) using 3 equiv of NBS.[6] We expected that this approach would lead to the electronically favored product **2** over **1**. Because the multiple products decomposed rapidly with storage and handling, only a preliminary separation was achieved with silica gel, without taking the mixture to dryness (thus, without quantitation of yield). Tetrabromopyrrole and other minor products were detected, but from ^{1}H and ^{13}C NMR spectral data, **2** appeared to represent ∼70% of the product mixture, and no chemical shift data suggestive of the presence of **1** were observed. NMR signals for products other than **2** were distinguished from those of **2** by HMQC and HMBC experiments (data not shown).

^{1}H and ^{13}C NMR spectral data for synthetic **1** were in close agreement with published data for **1** isolated from the hemichordate worm *Saccoglossus kowalevskii*.[1,4] In contrast, NMR spectral data for our synthetic **2** were clearly different than for synthetic and natural **1**. For example, for both natural[1] and synthetic **1**, the chemical shift of the only methine carbon (confirmed by DEPT and HMQC) was 121.9 ppm (in acetone-d_6) and 119.5 (in CDCl$_3$), whereas the methine carbon in synthetic **2** resonated at 114.1 ppm in acetone-d_6. The methine proton chemical shift was also consistent for natural[1] (δ 6.83) and

synthetic **1** (δ 6.81), whereas the methine proton of synthetic **2** resonated at δ 6.04 (all in CDCl$_3$).

Additional evidence supporting the identity of the natural product as 2,3,4-tribromopyrrole (**1**) came from spectral analysis of intermediate **5**. Strong NOEs were observed between H-5 and both the methyl and methine protons of the protecting group of 2,3,4-tribromo-1-(triisopropylsilyl)-pyrrole (**5**). Because ab initio calculations predicted a 2.1–2.5 Å separation of methine/methyl protons and H-5 for **5** and 4.8–5.2 Å separation if the pyrrole methine was at position 4, the observed NOEs favor the hypothesis that the pyrrole methine was adjacent to the nitrogen atom rather than at position 4 of the pyrrole system. Thus, it appears that a silyl protecting group did provide the steric bulk to overcome the electronic factors involved in determining regioselectivity of this electrophilic substitution reaction (resulting in bromination at positions 2, 3, and 4), whereas unprotected pyrrole (**3**), subjected to the same bromination reagent, was selectively transformed to the electronically favored product **2**. Taken together, the spectral data support our hypothesis that the tribrominated pyrrole natural product of *S. kowalevskii* is indeed 2,3,4-tribromopyrrole (**1**) and not 2,3,5-tribromopyrrole (**2**).

When predatory fish (*Fundulus heteroclitus*) were offered squid-based food pellets containing synthetic **1** at the concentration at which the natural product is found in *S. kowalevskii*, eight of 14 fish rejected pellets containing synthetic **1**, whereas all fish consumed control pellets (squid pellets without **1**), indicating a significantly deterrent effect of **1** ($p = 0.002$), similar to the deterrence of natural **1**.[1] The six fish that consumed the treated pellets appeared to be larger than the other eight, raising the possibility that larger individuals who generally eat more may be less sensitive to deterrent compounds. Thus, these six fish were fed control pellets to near-satiation and then were offered treated pellets a second time. Although three of these fish were too satiated to eat pellets of any kind in the second experiment, the remaining three fish rejected pellets treated with **1** and then consumed a control pellet, suggesting that the effectiveness of chemical defenses is affected by hunger status.

Compared to the deterrent properties of **1**, pyrrole (**3**) at the same concentration was palatable to *F. heteroclitus*, as all 12 fish offered pellets containing **3** consumed both treated and control pellets. This indicates that bromination plays a crucial role in the deterrence of **1**, although Kicklighter et al.[1] showed that many brominated aromatic compounds from marine worms do not deter predators at natural and greater than natural concentrations. Unfortunately, we were unable to test **2** due to its lability.

In conclusion, for the first time 2,3,4-tribromopyrrole (**1**) has been unambiguously and regioselectively synthesized. The NMR spectral data for synthetic **1** agree with previously reported data for **1** isolated from the marine worm *Saccoglossus kowalevskii*, whereas these data differ from that of synthetic **2**. Additionally, synthetic **1** was found to deter feeding by predatory fish.

Experimental Section

General Experimental Procedures. Melting points were measured with a Mettler Toledo FP62 melting point apparatus. NMR spectra were acquired using a Bruker Avance DRX 500, Bruker AMX 400, or Varian Mercury Vx 300 spectrometer, in CDCl$_3$, deteurioacetone, or deuteriodiethyl ether, and referenced to the residual light solvent. Low- and high-resolution mass spectra were acquired on a Micromass 70SE instrument with FAB and EI ionization. LC-MS data were generated using a HP Series 1100 system with electro-spray ionization, using a Symmetry C$_{18}$ column with a gradient mobile phase of acetonitrile and water. HPLC purification was performed with a Waters 515 pump and 2487 UV detector, using a Zorbax RX-SIL normal-phase column (10 × 250 mm). Molecular modeling was achieved by ab initio calculations with a Hartree–Fock database using a Spartan 2004 system. Solvents and other chemicals were purchased from Fisher Scientific and Sigma-Aldrich Corporation.

Bioassays. Feeding assays with the predatory fish *Fundulus heteroclitus* were performed in aquaria at the Georgia Institute of Technology's marine facility on Skidaway Island, Georgia, as previously described.[1] Consumption of treated and control foods were compared using a Fisher's exact test with an alpha value of 0.05.

***N*-(Triisopropylsilyl)pyrrole (4).** *n*-Butyllithium in hexane (19.3 mL of a 1.6 M solution, 30.8 mmol) was added dropwise to argon-dried pyrrole (**3**) (1.94 mL, 28.0 mmol) in distilled THF at −78 °C. Triisopropylsilyl chloride (6.00 mL, 28.0 mmol) was added after 10 min and the reaction warmed to room temperature. The solvent was then removed, water was added, and the resulting residue was extracted with diethyl ether. The organic phase was then dried over anhydrous magnesium sulfate and concentrated by rotary evaporation. *N*-(Triisopropylsilyl)pyrrole (**4**) was isolated as a colorless oil (6.26 g, 28.0 mmol), in 100% yield: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.79 (2H, t, $J = 1.8$), 6.31 (2H, t, $J = 2.0$), 1.44 (3H, septet, $J = 7.6$), 1.09 (18H, d, $J = 7.2$); ^{13}C NMR (100 MHz) δ 124.0, 110.0, 17.8, 11.7; FABMS m/z 224.2 (15), 223.2 (20), 180.2 (11), 153.2 (25), 154.2 (100), 137 (29), 136 (57), 138 (65), 106.6 (19); HRFABMS [M]$^+$ m/z 223.1765 (calc for C$_{13}$H$_{25}$NSi 223.1756).

2,3,4-Tribromo-1-(triisopropylsilyl)pyrrole (5). *N*-(Triisopropylsilyl)pyrrole (**4**) (2.00 g, 9.00 mmol) was dissolved in distilled THF (5.0 mL) and cooled to −78 °C. NBS (4.78 g, 26.9 mmol) in THF (35 mL) was then added over 10 min, and the reaction was warmed to room temperature overnight under argon. Cold hexane was then added to the reaction mixture to precipitate unreacted NBS and succinimide byproduct, and the slurry was filtered through neutral alumina. The partially brominated intermediate was dissolved in distilled THF (5.0 mL) and cooled to −78 °C. NBS (2.40 g, 13.5 mmol) in THF (20 mL) was added as before and the product recovered as above. Solvent was removed by rotary evaporation, and the product was filtered through silica gel and recrystallized in pentane at −78 °C to produce **5** as a pale yellow solid (4.00 g, 8.69 mmol) in 97% yield: mp 50 °C; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.85 (1H, s), 1.66 (3H, septet, $J = 7.6$), 1.12 (18H, d, $J = 7.6$); ^{13}C NMR (100 MHz) δ 125.7, 105.6, 104.6, 100.8, 34.1, 18.0; EIMS m/z 464.9 (32), 462.9 (88), 460.9 (87), 458.9 (30), 419.9 (9), 417.9 (24), 415.9 (25), 413.9 (9), 382 (26), 380 (47), 378 (25), 204.9 (9), 202.9 (22), 200.9 (9), 157.1 (95), 138.0 (39), 136.0 (38), 115.1 (100), 87.1 (52), 73.0 (47), 59.0 (58); HREIMS [M]$^+$ m/z 458.9055 (calc for C$_{13}$H$_{22}$Br$_3$NSi 458.9051).

2,3,4-Tribromopyrrole (1). 2,3,4-Tribromo-1-(isopropylsilyl)pyrrole (**5**) (100 mg, 0.217 mmol) was dissolved in THF (3.0 mL) at room temperature. TBAF (0.543 mL, 0.543 mmol) as a solution in THF was slowly added, and the mixture stirred for approximately 1 h. The mixture was then washed with water (3×), sodium bicarbonate (3×), and brine (3×). The organic materials were concentrated in an ice bath with a stream of nitrogen gas to yield **1** as a pale yellow oil (59.3 mg; 0.195 mmol) in 90% yield: ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.81 (1H, br s), 6.81 (1H, d, $J = 3.2$); ^{1}H NMR (acetone-d_6, 400 MHz) δ 11.23 (1H, br s), 7.11 (1H, d, $J = 3.2$); ^{1}H NMR (diethyl ether-d_{10}, 500 MHz) δ 11.05 (1H, br s), 6.87 (1H, s); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 119.5 (CH), 101.9 (C), 100.2 (C), 99.8 (C); ^{13}C NMR (acetone-d_6, 100 MHz) δ 121.9 (CH), 101.7 (C), 101.2 (C), 99.5 (C); EIMS m/z 306.8 (34), 304.8 (98), 302.8 (99), 300.8 (37), 225.9 (14), 223.9 (28), 221.9 (15), 198.9 (9), 196.9 (20), 194.9 (10); HREIMS [M]$^+$ m/z 300.7749 (calc for C$_4$H$_4$Br$_3$N 300.7737).

2,3,5-Tribromopyrrole (2). NBS (1.92 g, 10.8 mmol) in THF (10 mL) was added over a period of 10 min to a solution of pyrrole (**3**) (242 mg, 3.60 mmol) stirring in THF (10 mL) under nitrogen, at −78 °C. After 10 more minutes, the reaction

mixture was warmed to −10 °C for 2 h, then warmed to room temperature, and most of the THF was removed by rotary evaporation. A portion of the product mixture was separated on silica gel using a gradient of hexane and diethyl ether. Iterative evaporation of solvent using a gentle N_2 stream and addition of NMR solvent to portions of the product mixture that had and had not been subjected to silica gel separation enabled isolation of **2** for spectral analysis without exposing the product to dryness. Further attempts to store the product or purify it by HPLC resulted in rapid decomposition. ^{1}H NMR of **2** (within a mixture that contained ∼70% **2** plus tetrabromopyrrole and other minor products): (CDCl$_3$, 500 MHz) δ 6.04 (d, J = 2.9); ^{1}H NMR (acetone-d_6, 500 MHz) δ 11.55 (1H, br s), 6.24 (1H, d, J = 2.8); ^{13}C NMR (acetone-d_6, 100 MHz) δ 114.1 (CH), 100.3 (C), 99.1 (C), 99.0 (C); EIMS m/z 306.8 (38), 304.8 (99), 302.8 (100), 300.8 (36), 225.9 (13), 223.9 (26), 221.9 (13), 198.7 (11), 196.9 (21), 194.9 (10); HREIMS [M]$^+$ m/z 300.7763 (calc for C$_4$H$_4$Br$_3$N 300.7737).

References and Notes

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